MARTIN et al.

1, 21, 42, 43, 44, 51

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Martin *et al.*
Serial No. 10/026,302

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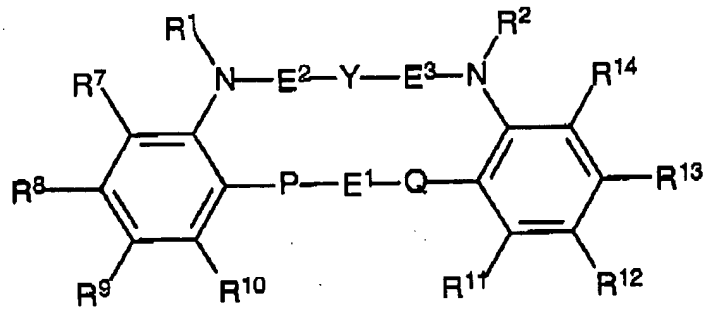
What is claimed is:

1. (Currently Amended) A compound of the formula



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

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Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, a biologically compatible esterifying group, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive [functional] group that is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, or $-(C(O)CH_2)_n-$,where n = [2-4] 2, 3 or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 and R^2 are independently [H; or] -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{16}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$;

R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE, -L-R_X, or -L-S_C at R^1, R^2, R^4, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13}, or R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE.

2. (Original) A compound, as claimed in Claim 1, wherein each R^5 is H and each n is 2.

3. (Original) A compound, as claimed in Claim 1, wherein Y is NR^4.

4. (Original) A compound, as claimed in Claim 1 wherein P and Q are O.

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5. (Original) A compound, as claimed in Claim 4, wherein Y is O.

6. (Original) A compound, as claimed in Claim 5, wherein said compound is substituted by only one -L-R_X, or -L-S_C, that is bound at R^8, R^9, R^{12}, or R^{13}.

7. (Original) A compound, as claimed in Claim 1, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$.

8. (Original) A compound, as claimed in Claim 1, wherein R^8 and R^9, and optionally R^{12} and R^{13}, taken in combination, form a fused DYE that is a substituted or unsubstituted benzofuran.

9. (Original) A compound, as claimed in Claim 1, wherein said compound is substituted by exactly two DYE or fused DYE moieties.

10. (Currently Amended) A compound, as claimed in Claim 1, wherein said compound is substituted by exactly one -L-DYE moiety at R^9, and <u>said compound</u> is optionally [additionally] substituted <u>at a position other than R^9</u> by exactly one -L-R_X or exactly one -L-S_C.

11. (Original) A compound, as claimed in Claim 1, wherein each L is independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination

of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

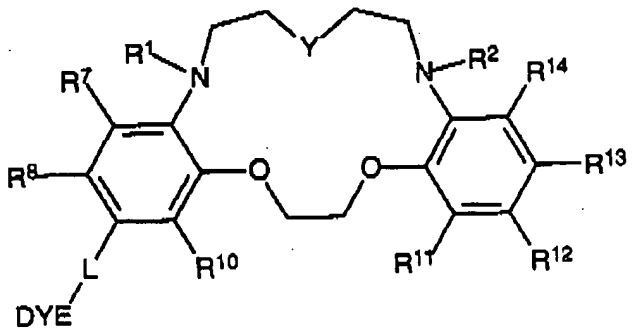
12. (Original) A compound, as claimed in Claim 11, wherein L is a single covalent bond or has the formula $-(CH_2)_d(CONH(CH_2)_e)_z-$ or $-O(CH_2)_d(CONH(CH_2)_e)_z-$, where d is an integer from 0-5, e is an integer from 1-5 and z is 0 or 1.

13. (Currently Amended) A compound, as claimed in Claim 1, that is substituted by at least one S_c[, wherein each S_c is] selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, [a psoralen,] a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, a biotin, a silica [or] and a virus.

14. (Currently Amended) A compound, as claimed in Claim 13, wherein said compound is substituted by exactly one S_c that is a protein, a polysaccharide, a biotin, or a silica.

15. (Currently Amended) A compound, as claimed in Claim 1, that is substituted by at least one –L- R_x[, wherein R_x is] selected from the group consisting of a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide, an aliphatic amine, a silyl halide, [or] and a psoralen.

16. (Original) A compound, as claimed in Claim 1, having the formula



wherein Y is O or NR^4.

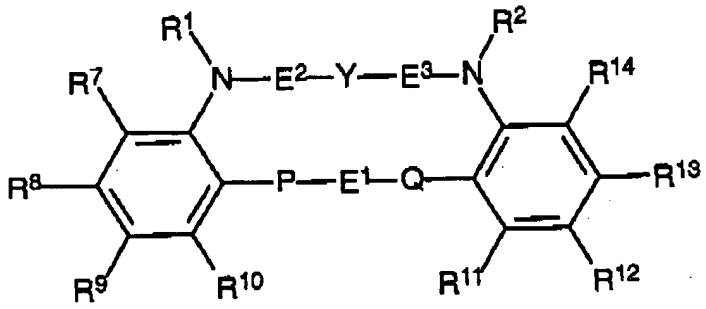
17. (Original) A compound, as claimed in Claim 16, wherein DYE is an indole, a coumarin, a stilbene, a xanthene, an oxazine, or a polyazaindacene.

18. (Original) A compound, as claimed in Claim 17, wherein DYE is a fluorescein, a rhodamine, a rhodol, a polyazaindacene, an oxazine, a 3*H*-xanthen-6-ol-3-one, a 6-amino-3*H*-xanthen-3-one, or a 6-amino-3*H*-xanthen-3-imine, and L is a single covalent bond.

19. (Original) A compound, as claimed in Claim 16, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$.

20. (Original) A compound, as claimed in Claim 19, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16}, where each R^{16} is H, C_1-C_6 alkyl, an alpha-acyloxymethyl, a t-butyldimethyldimethylsilyl, or a biologically compatible salt.

21. (Currently Amended) A composition of matter comprising a compound of the formula:



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally

substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{15}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, a biologically compatible esterifying group, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

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each L is independently a covalent linkage;

each R_X is independently a reactive [functional] group;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently -$(CR^5_2)_n$-, where n = [2-4] 2, 3, or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 and R^2 are independently [H; or] -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, -(C=O)-$NR^{17}R^{18}$;

R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally

substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE moiety at one or more of R^1, R^2, R^3, and R^7-R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE.

22. (Original) A composition, as claimed in Claim 21, wherein each R^5 of the compound is H and each n is 2.

23. (Currently Amended) A composition, as claimed in Claim 21, wherein each R_X of the compound is independently a reactive [functional] group that is an acrylamide, an activated ester of a carboxylic acid, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, or a thiol.

24. (Currently Amended) A composition, as claimed in Claim 21, wherein each P and Q [on the compound] are O.

25. (Currently Amended) A composition, as claimed in Claim 24, wherein each Y [on the compound] is O.

26. (Original) A composition, as claimed in Claim 25, wherein said compound is substituted by only one -L-R_X, or -L-S_C, that is bound at R^6, R^9, R^{12}, or R^{13}.

27. (Original) A composition, as claimed in Claim 25, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$.

28. (Original) A composition, as claimed in Claim 21, wherein R^8 and R^9, and optionally R^{12} and R^{13}, taken in combination, form a fused DYE that is a substituted or unsubstituted benzofuran.

29. (Original) A composition, as claimed in Claim 21, wherein said compound is substituted by exactly two DYE or fused DYE moieties.

30. (Currently Amended) A composition, as claimed in Claim 21, wherein said compound is substituted by exactly one -L-DYE moiety at R^9, and <u>said compound</u> is optionally [additionally] substituted by exactly one -L-R_X or exactly one -L-S_C <u>at a position other than R^9</u>.

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31. (Original) A composition, as claimed in Claim 21, wherein each L of the compound is independently a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

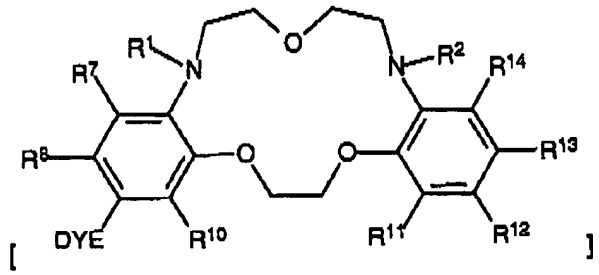
32. (Original) A composition, as claimed in Claim 31, wherein each L of the compound is a single covalent bond or has the formula -$(CH_2)_d(CONH(CH_2)_e)_z$- or -$O(CH_2)_d(CONH(CH_2)_e)_z$-, where d is an integer from 0-5, e is an integer from 1-5 and z is 0 or 1.

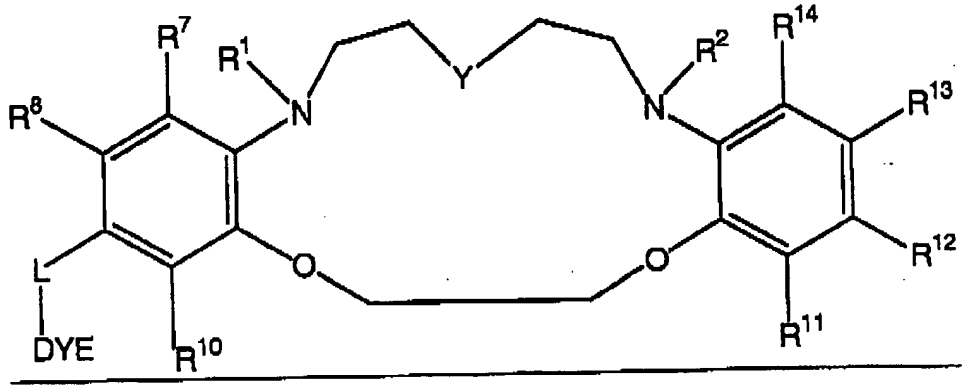
33. (Currently Amended) A composition, as claimed in Claim 21, wherein each S_C of the compound is an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, [a psoralen,] a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell, <u>a biotin, a silica</u> or a virus.

34. (Currently Amended) A composition, as claimed in Claim [21] 33, wherein said compound is substituted by exactly one S_C, which S_C is a protein, a polysaccharide, a biotin, or a silica.

35. (Currently Amended) A composition, as claimed in Claim 21, wherein said compound is substituted by exactly one R_X[, which R_X is] selected from the group consisting of a succinimidyl ester of a carboxylic acid, a haloacetamide, a hydrazine, an isothiocyanate, a maleimide, an aliphatic amine, a silyl halide, [or] and a psoralen.

36. (Currently Amended) A composition, as claimed in Claim 21, where the compound has the formula:





[where R^1, R^2, R^7, R^8, and R^{10} are not –L-DYE, and no more than one, and optionally none, of R^{11}-R^{14} is –L-DYE] wherein Y is O or NR^4.

37. (Original) A composition, as claimed in Claim 36, wherein each DYE on the compound is a fluorescein, a rhodamine, a rhodol, a polyazaindacene, an oxazine, a

3*H*-xanthen-6-ol-3-one, a 6-amino-3*H*-xanthen-3-one, or a 6-amino-3*H*-xanthen-3-imine.

38. (Original) A composition, as claimed in Claim 36, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$.

39. (Original) A composition, as claimed in Claim 38, wherein R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by -(C=O)-O-R^{16}, where each R^{16} is H, an alpha-acyloxymethyl, a t-butyldimethyldimethylsilyl, or a biologically compatible salt.

40. (Original) A composition, as claimed in Claim 36, further comprising a metal ion that is Ca^{2+}, Na^+, K^+, or Zn^{2+} associated with said compound.

41. (Original) A composition, as claimed in Claim 21, further comprising a natural or synthetic polymer or glass.

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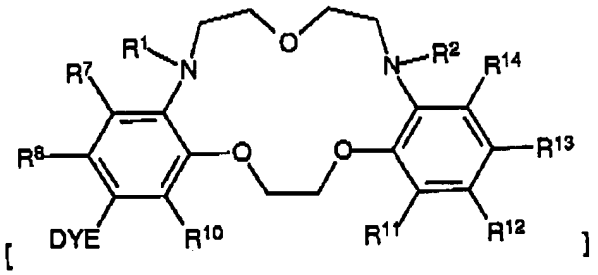
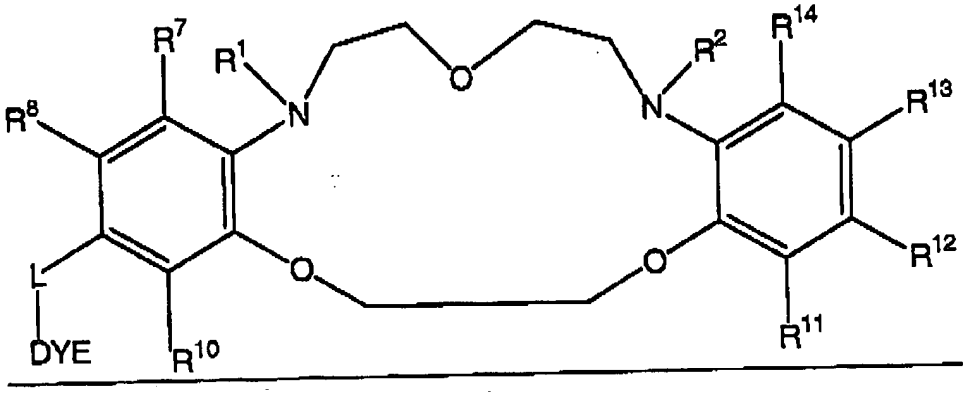
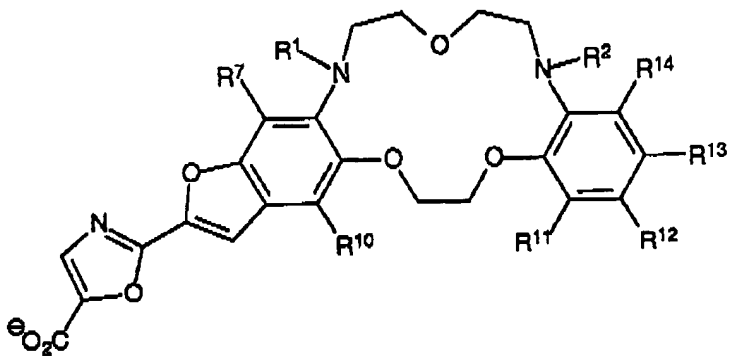
42. (Currently Amended) A compound having the formula:





or the formula:



wherein

R^1 and R^2 are C_1-C_6 alkyl that are substituted one or more times by cyano, an aryl or heteroaryl ring system, or by -(C=O)-O-R^{18} or -(C=O)-$NR^{17}R^{18}$, where

R^{18} is H, a C_1-C_6 alkyl, a benzyl, a biologically compatible esterifying group, or a biologically compatible salt;

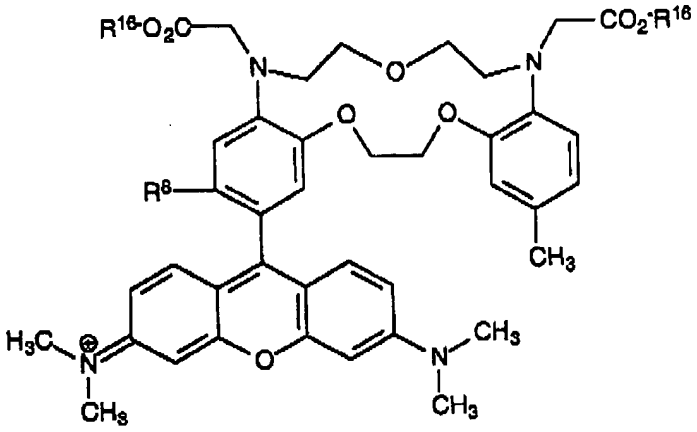
R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxymethyl, or a biologically compatible salt;

R^7-R^{10}, and R^{11}-R^{14}, where present, are independently H, chloro, bromo, fluoro, nitro, amino, or cyano; or C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{16}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; [and]

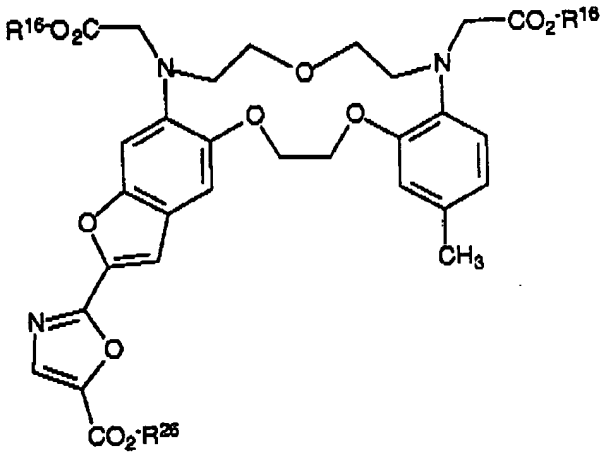
L is a covalent linkage; and

DYE, where present is a polyazaindacene, an oxazine, or a xanthene, which is optionally substituted by halogen, nitro, sulfo, cyano, an aryl or heteroaryl ring system, or benzo, or alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, the alkyl portions of which contain fewer than 20 carbons.

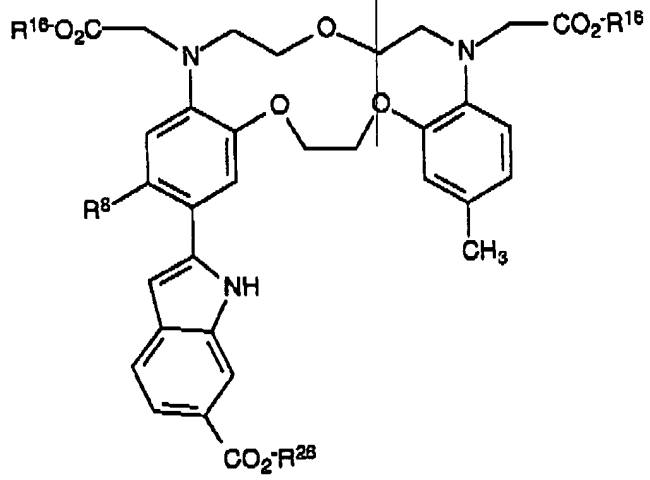
43. (Original) A compound having the formula:



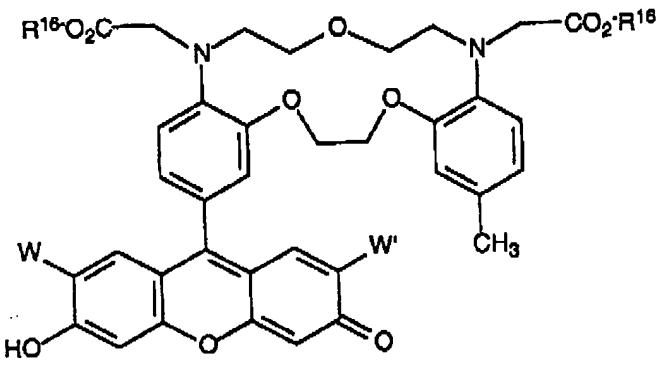
or the formula



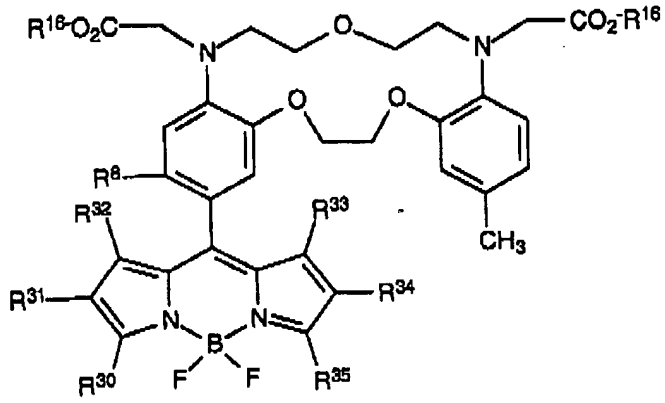
or the formula:



or the formula:



or the formula:



wherein

R^8, where present, is independently H or a C_1-C_6 alkyoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{26}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

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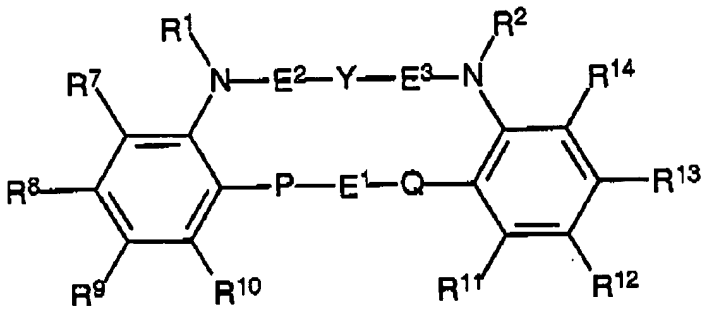
R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system; or adjacent substituents R^{31} and R^{32}, and adjacent substituents R^{33} and R^{34}, when taken in combination form a fused benzo ring that is optionally substituted by hydrogen, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, alkylthio, alkylamido, amino, monoalkylamino or dialkylamino wherein the alkyl portions of each contain fewer than 20 carbons.

44. (Currently Amended) A method of detecting a target cationic metal ion in a sample, comprising:

a) adding to said sample, in an amount sufficient to generate a detectable optical response when said target ion is present, a compound having the formula:



wherein

P and Q are independently O, S, or NR^3, where each R^3 is independently H or C_1-C_6 alkyl;

Y is O, S, or NR^4, where R^4 is H; or is -L-R_X, -L-S_C, or -L-DYE; or is C_1-C_{18} alkyl or an aryl or heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, or -L-DYE; or by C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is H or C_1-C_6 alkyl; or -L-R_X, -L-S_C, or -L-DYE;

R^{16} is H, a C_1-C_6 alkyl, a benzyl, a biologically compatible esterifying group, a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE;

R^{17} and R^{18} are independently H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt; or -L-R_X, -L-S_C, or -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive [functional] group;

each S_C is independently a conjugated substance;

DYE is a chemical moiety with an absorption maximum beyond 320 nm;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, where n = [2-4] 2, 3, 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered allphatic ring;

R^1 and R^2 are independently [H; or] -L-R_X, -L-S_C, or -L-DYE; or C_1-C_{18} alkyl or C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, or by an aryl or heteroaryl ring system; or by $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$; or by C_1-C_6 alkylamino, C_2-C_{12} dialkylamino; or by C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$;

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R^7-R^{14} are independently H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L--S_C, -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, or -L-DYE; or C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{14}, taken in combination with each other, and with the aromatic ring they are bound to, form a fused DYE;

provided that said compound is substituted by at least one -L-DYE moiety at one or more of R^1, R^2, R^3, and R^7-R^{14}; or at least two of R^7-R^{14}, taken in combination, form a fused DYE;

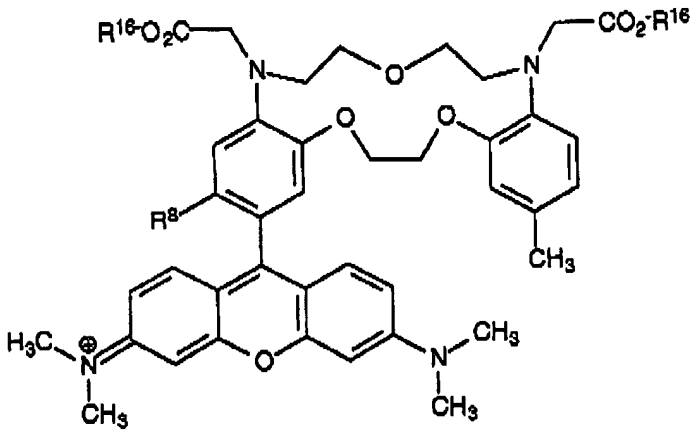
b) illuminating said sample to generate said detectable optical response [that indicates that] whereby said target ion is present.

45. (Original) A method, as claimed in Claim 44, wherein said detectable optical response is a fluorescence response.

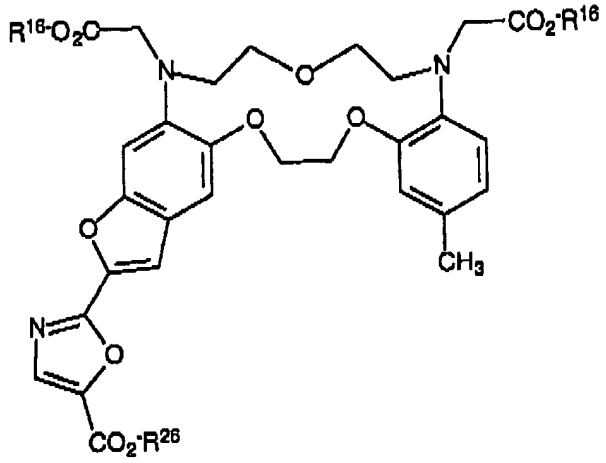
46. (Currently Amended) A method, as claimed in Claim 45, wherein [the step of] said illuminating is performed in conjunction with a fluorometer, fluorescence microscope, laser scanner, flow cytometer, a microfluidic device, or a fiber optic probe.

47. (Original) A method, as claimed in Claim 44, wherein said target metal ion is Na^+, K^+, Ca^{2+}, or Zn^{2+}.

48. (Original) A method, as claimed in Claim 44, wherein said compound has the formula:



or the formula:



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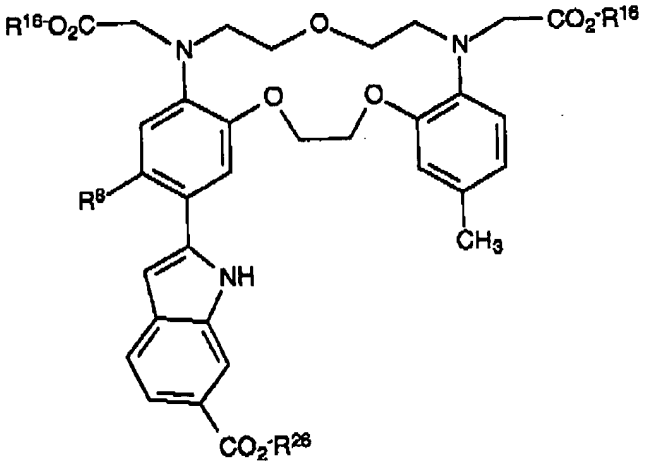
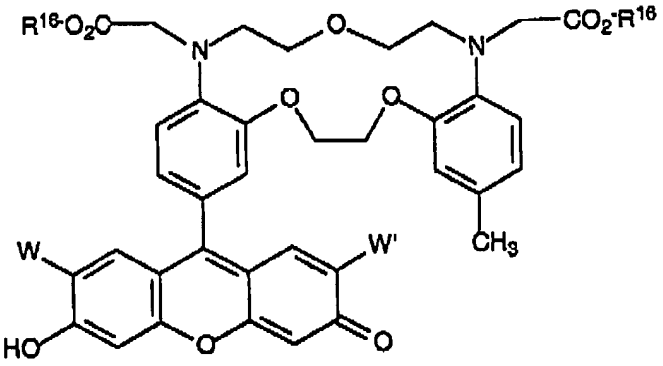
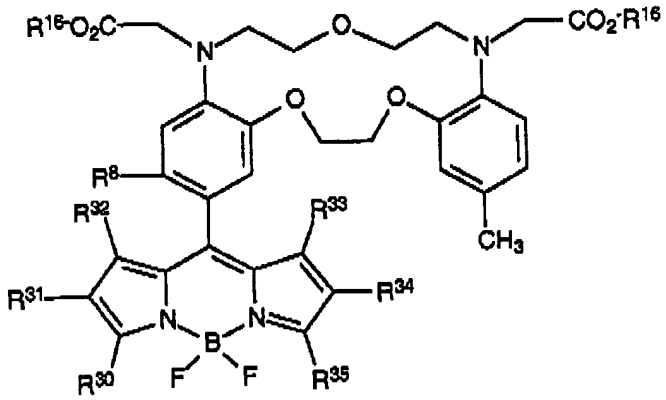
or the formula:



or the formula:



or the formula:



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wherein

R^8, where present, is independently H or a C_1-C_6 alkyoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{26}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

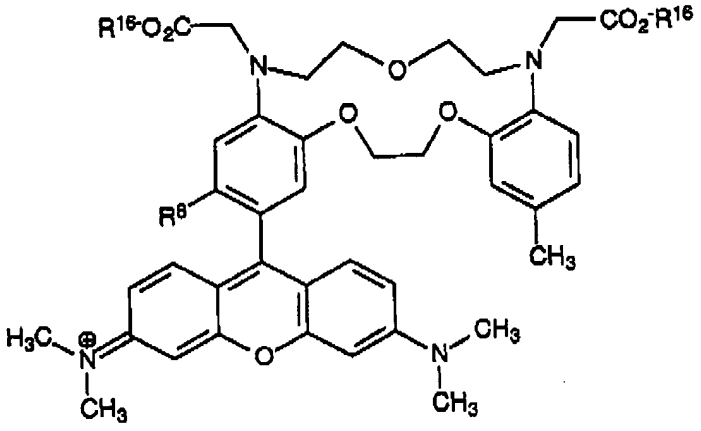
R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the

alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system.

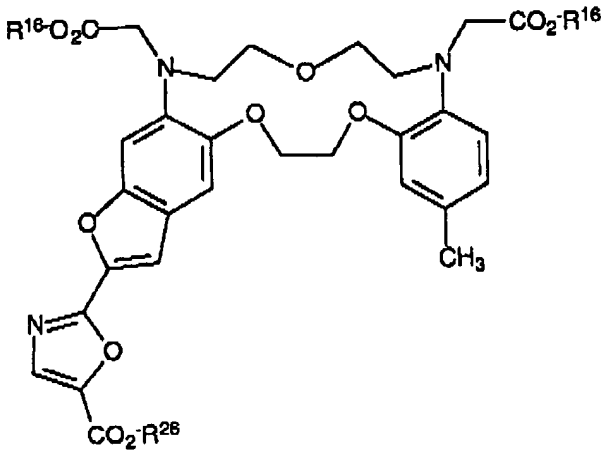
49. (Original) A method, as claimed in Claim 48, wherein said target metal ion is Na^+ or K^+.

50. (Currently Amended) A method, as claimed in Claim 44, wherein said sample [further] comprises living cells or biological fluids.

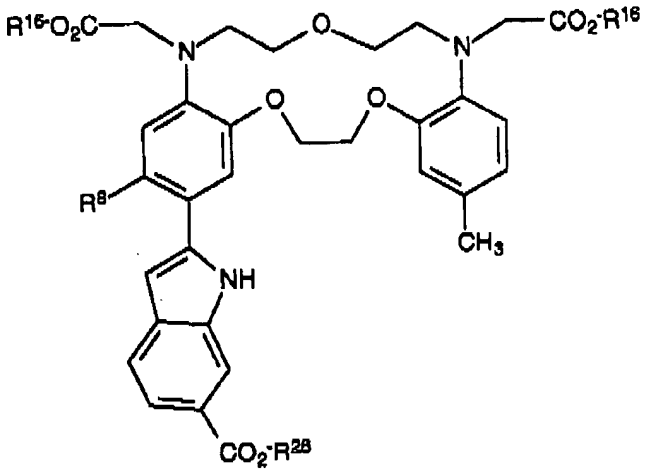
51. (Original) A kit for the detection or quantification of a target metal ion, comprising a compound having the formula:



or the formula:



or the formula:



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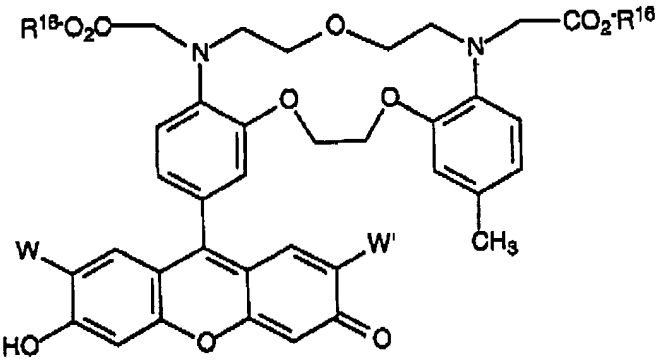
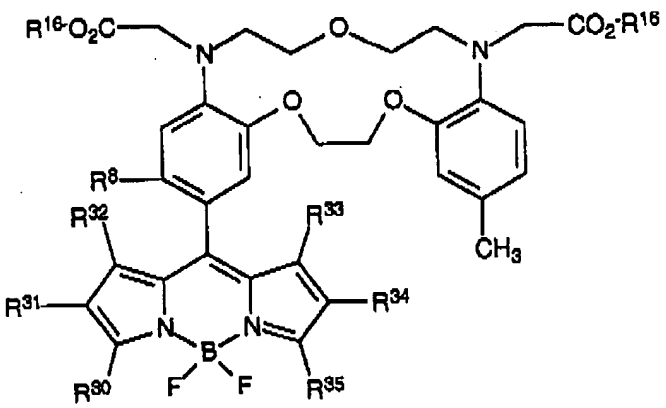
or the formula:



or the formula:



wherein

R^8, where present, is independently H or a C_1-C_6 alkyoxy, which is optionally substituted by -(C=O)-O-R^{16} or -(C=O)-$NR^{17}R^{18}$;

R^{16} and R^{26}, where present, are independently H, a C_1-C_6 alkyl, a benzyl, an alpha-acyloxyalkyl, a t-butyldimethylsilyl, or a biologically compatible salt;

R^{17} and R^{18}, where present, are independently H, a C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, or a biologically compatible salt;

W and W', where present, are independently F or Cl;

R^{30}-R^{35}, where present, are independently H, halogen, nitro, sulfo, cyano, alkyl, perfluoroalkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, arylalkyl, or acyl, wherein the alkyl portions of each contain fewer than 20 carbons; or an aryl or heteroaryl ring system;

and comprising one or more components selected from the group consisting of:

a) a calibration standard of a target ion;

b) an ionophore;

c) a fluorescence standard;

d) an aqueous buffer solution; and

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e) an organic solvent.

Respectfully submitted,

Date: June 16, 2003

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